DM REVIEW

Autoimmunity in Diabetic Autonomic Neuropathy: Does the Immune System Get On Your Nerves?

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Symptomatic autonomic neuropathy is a devastating occasional complication of diabetes mellitus, especially Type 1. Although the full-blown clinical syndrome is not common, dysfunction of the autonomic nerves is detectable in up to 40 % of Type 1 diabetic patients but its aetiopathogenesis is poorly understood. There is evidence to suggest that the damage to the autonomic nerves may be immune-mediated. This evidence is reviewed in the following article. © 1998 John Wiley & Sons, Ltd.

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Introduction

Autonomic dysfunction is detectable in up to 40 % of diabetic patients.[1,2] Only a small proportion of these develop clinical symptoms and signs, such as gastroparesis, postural hypotension, gustatory sweating, diabetic diarrhoea and bladder paresis.[3] For the patient, symptomatic autonomic neuropathy is undoubtedly one of the most unpleasant and severe late complications of diabetes mellitus (DM), occasionally leaving patients completely crippled and debilitated;[3] for the diabetologist, diabetic autonomic neuropathy (DAN) is perhaps one of the more difficult complications to understand and manage. Moreover, the development of autonomic failure significantly worsens prognosis.[4,5]

It seems probable that the overall causative mechanism is multifactorial and complex in nature, a view supported by the heterogeneity of the clinical presentation. The manifestations of diabetic neuropathy vary greatly from one patient to the other, and some patients with abnormal autonomic function tests may escape all symptoms, while others become gravely affected after a few years of diabetes.

A number of pathogenetic mechanisms for diabetic autonomic neuropathy (DAN) has been suggested, as for the other diabetic neuropathies: local ischaemia leading to nerve fibre death;[6,7] persistent hyperglycaemia resulting in activation of the polyol pathway and tissue accumulation of sorbitol, fructose, and deficiency of myoinositol, and also in perturbed phosphoinositide metabolism with subsequently decreased nerve Na^+/K^+ATPase activity;[8–10] increased non-enzymatic glycation; decreased nitric oxide production leading to impaired endothelium-dependent vasodilation and, again, Na^+/K^+ATPase activity;[10] and, finally, deficiency in neurotrophic growth factors.[11–12] However, in addition to these mechanisms, there is now growing evidence that DAN is characterized by immune system involvement, and the discussion and debate of these reports will form the major part of this review. Two questions will inevitably arise, and will need to be addressed in the next decade of studies: does the immune system actually mediate damage to autonomic nervous tissue (ANT); and are autoimmune phenomena diagnostic or predictive of DAN?

As contributors to, and followers of, the current literature on autoimmunity and DAN, we have based much of this review on our knowledge of previous publications. In addition, we have carried out MEDLINE searches based on appropriate key words.

In immunological terms, the investigation of DAN is not particularly well advanced by comparison with other putative autoimmune diseases, such as Type 1 DM itself. However, immunology and immunological techniques have made rapid advances. In an attempt to make this article more accessible, a limited overview and glossary has been provided (Table 1).

Abbreviations: ANT autonomic nervous tissue, CF-ADM complement-fixing adrenal medullar autoantibodies, CF-SG complement-fixing cervical sympathetic ganglia autoantibodies, CF-VN complement-fixing vagus nerve autoantibodies, CPH carboxypeptidase H, DAN diabetic autonomic neuropathy, GAD glutamic acid decarboxylase, IFL immunofluorescence

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Table 1. Glossary of immunological terms

Cell type	Function
Macrophage or dendritic cell	Termed antigen presenting cells (APC). Ingest and digest complex protein antigens or autoantigens, and present small fragments embedded in HLA class II molecules to CD4+ T cells
CD4+ T lymphocyte	Responds to antigen presented by APC, via specific surface receptor (analogous to antibody). Major cell type responsible for generating immune responses through recruitment of other effector cells
CD8+ T lymphocyte	Recruited by CD4+ T cells to regulate immune response, or kill target cells. Also has antigen-specific surface receptor
B lymphocyte	Recruited by CD4+ T cells to make specific antibody responses
Cytokines	Small molecular weight, soluble factors released by immune cells to communicate with and influence the function of other cells through specific surface receptors

Background for the Immune Hypothesis in DAN

The first indication of immune involvement in DAN arose from post-mortem studies of five patients with Type 1 DM and symptomatic autonomic neuropathy. Autonomic ganglia and autonomic nerve bundles were heavily infiltrated by small lymphocytes, plasma cells and macrophages,[13] with an accompanying destruction of the normal ganglion architecture and loss of tissue (Figure 1). A more recent post mortem-examination of a patient with severe DAN highlighted a striking atrophy of the cervical sympathetic ganglia with prominent cell loss.[14] The ganglia again displayed infiltration by lymphocytes and macrophages. Formal studies on the exact composition of this infiltrate, and in particular the type of lymphocyte (T or B; if T then CD4 or CD8) have not been done. However, much can be inferred from this pathological picture, since, although this type of aggregation is seen in immune responses to viruses or intracellular bacteria, it is also characteristic of autoimmune destruction: similar aggregation is seen in the islets in Type 1 DM,[15] in Addisonian adrenalitis,[16] and in autoimmune oophoritis.[17] A similar pathological entity has been seen in the adrenal medulla ('adrenal medullitis') in a significant proportion of long-standing Type 1 DM patients.[14,18] It is strongly suggestive of a pathological scenario in which tissue macrophages present self- antigens to autoreactive CD4+ T lymphocytes. Activated CD4+ T lymphocytes and macrophages can cause tissue damage themselves, through release of cytokines (interferon-γ, interleukin-1, tumour necrosis factor-α) or through recruitment of other effectors: B cells to make antibody which can be damaging and CD8+ T lymphocytes which can kill target cells directly. Of course, it can be argued that the collection of cells seen at the nerve-side is peripheral or secondary to whatever is causing the nerve damage. However, whatever causes it to appear, these cells could further damage the nervous system by cytokine release or direct toxicity.

Although there are occasional reports of lymphocytic infiltrates in blood vessel walls, perivascular spaces and endoneurium in all the clinical forms of diabetic neuropathy,[19–21] it is noteworthy that lymphocytic infiltration does not appear to be a consistent feature of peripheral somatic neuropathies in Type 1 DM, one of a number of contrasting pathological features between it and DAN, and arguing strongly for the direct relevance of the infiltrate in the latter.

The presence of plasma cells (end-differentiated B cells which act as antibody factories) in the infiltrate supports the scenario expanded above, since such B cell development is entirely dependent upon the active influence of CD4+ T lymphocytes. At the same time, it is consistent with a process that is more chronic than acute. In summary, an argument can be made for the cellular infiltrate representing an autoimmune response to ganglion cells. In this context, and as a direct consequence of B cell differentiation, one might expect the generation of autoantibodies related to the pathological process taking place in the ganglia.

Autonomic Nervous System Autoantibodies

From the mid-1970s onwards, after the first description of autoantibodies in a human organ-specific autoimmune disease (anti-thyroglobulin autoantibodies in autoimmune thyroiditis), there has been considerable interest in establishing the same phenomenon in other conditions. One of the most successful approaches has been indirect immunofluorescence (IFL). Normal tissue from the putative target organ is acquired fresh and deep frozen, cut into thin sections and then incubated with patient serum. After washing, binding of potential autoantibodies is revealed either with an anti-human IgG antiserum, or an anti-human complement antiserum, in which case the autoantibodies are required to bind complement after binding to target in the tissue (both antisera are raised in rabbits and conjugated to a fluorescent tag). In the late 1980s, the same procedure was used to search for autoantibodies to tissues deriving from the autonomic nervous system, namely sympathetic ganglion, parasympathetic nerve (both of rabbit origin), and adrenal medulla (usually human or primate origin). At present these tissues remain the substrates most widely used for the detection of anti-autonomic nervous tissue (ANT) autoantibodies.

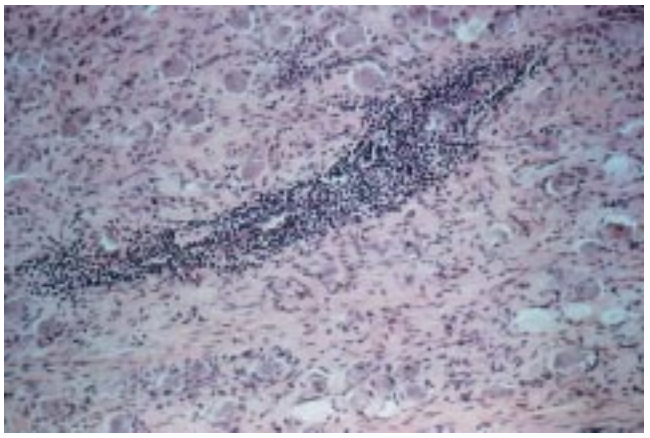

Figure 1. Post-mortem section of sympathetic ganglion from a patient with severe diabetic autonomic neuropathy, showing dense infiltrate of lymphocytes, macrophages and plasma cells

In the first such studies, complement-fixing anti-adrenal medulla (CF-ADM) autoantibodies were detected in a high proportion of newly diagnosed Type 1 DM patients.[22] This observation was followed by several studies from Rabinowe's group, and then from others, with a broadening of the research leading to the identification of complement-fixing autoantibodies reacting with cytoplasmic targets in the cell bodies in cervical sympathetic ganglia (CF-SG), and others reactive with targets within fibres of vagus nerve bundles (CF-VN) (Figure 2). The major message from these studies, summarized in Table 2, is that these autoantibodies are present at a higher prevalence in Type 1 DM patients than in healthy controls. To our knowledge, the disease specificity has yet to be explored more formally with studies of thyroid or other autoimmune diseases. However, the nervous tissue autoantibodies are not associated with the presence of other organ-specific autoantibodies (islet cell, thyroglobulin and microsomal antibodies) in patients with Type 1 DM.[24] Anti-adrenal medulla autoantibodies are quite common at diagnosis of Type 1 DM, with lower prevalences in Type 1 DM of short or long duration. The prevalence CF-SG at diagnosis is high according to one report (40 %),[27] while that of CF-VN has not been reported in the literature. Preliminary evidence from a large cross-sectional study ($n = 394$) of these autoantibodies in Type 1 DM of varying duration supports the proposal that CF-ADM are high near to diagnosis and then wane, whereas CF-SG and CF-VN have a low prevalence near to diagnosis of Type 1 DM and increase in prevalence with diabetes duration (Ejskjaer *et al.*, unpublished). Further studies will be required to settle this controversy. It would appear that ANT autoantibodies tend, in the majority of cases, to occur independently; multiple autoantibodies present in an individual is unusual.

Finally, there is evidence that all three autoantibodies may be found in first-degree relatives of Type 1 DM patients.[25,29] Although these studies were carried out in the years before antibody-based prediction of Type 1 DM in high risk groups was as refined as it is now, some of the first degree relatives studied displayed evidence of possible risk of Type 1 DM themselves (positivity for islet cell antibody, ICA), indicating that the autoantibodies may be present in a proportion of cases

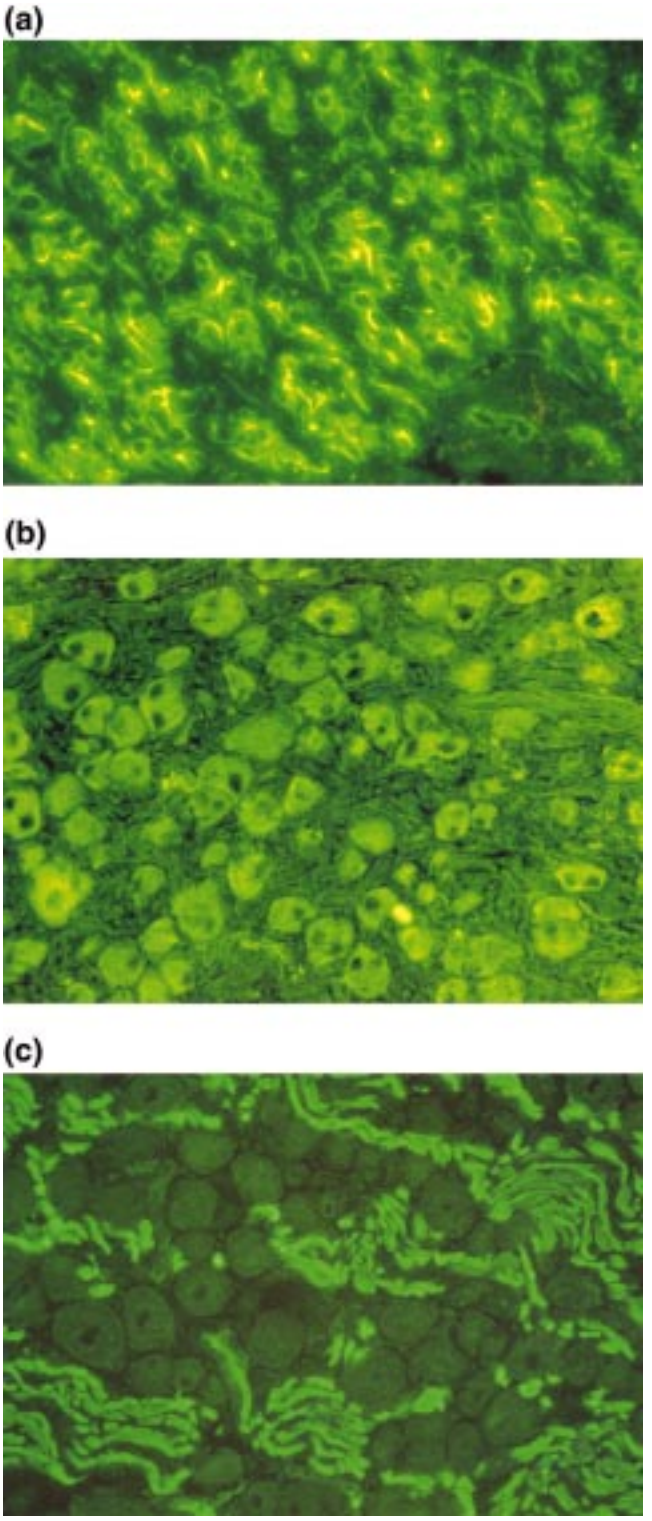

Figure 2. Immunofluorescence studies (complement-fixing) using sera from patients with diabetic autonomic neuropathy, staining (a) adrenal medulla, (b) sympathetic ganglion and (c) vagus nerve

Table 2. Review of prevalence of anti-nervous tissue autoantibodies in Type 1 DM

Autoantibody	Study group	Prevalence (%) (*n*)
Complement-fixing adrenal medulla	Type 1 DM at diagnosis	21 (43)[22]
	Short duration Type 1 DM (0–16 years)	19 (62)[23] 13 (15)[24]
	Long duration Type 1 DM (>16 years)	3 (32)[23] 8 (25)[24a]
	Long duration Type 1 DM with DAN	13 (37)[24]
	Type 1 DM varying duration (0–30 years)	17 (47)[25]
	Healthy controls	6 (32)[25] 3 (70)[26] 3 (27)[24]
Complement-fixing sympathetic ganglia	Type 1 DM at diagnosis	40 (20)[27]
	Short duration Type 1 DM (0–16 years)	13 (15)[24]
	Long duration Type 1 DM (>16 years)	0 (25)[24a] 18 (55)[28]
	Long duration Type 1 DM with DAN	20 (37)[24]
	with CAN	34 (41)[28]
	Type 1 DM varying duration (0–43 years)	22 (94)[29]
	Healthy controls	0 (27)[24] 5 (89)[28]
Complement-fixing vagus nerve	Short duration Type 1 DM (0–16 years)	13 (15)[24]
	Long duration Type 1 DM (>16 years)	0 (25)[24a]
	Long duration Type 1 DM with DAN	10 (37)[24]
	Type 1 DM varying duration (0–43 years)	8 (83)[30]
	Healthy controls	0 (27)[24] 0 (76)[26]

[a]Long-standing Type 1 DM patients in this study all had normal autonomic function tests.
CAN, cardiac autonomic neuropathy.

before diagnosis of diabetes. It is also noteworthy that ANT autoantibodies are relatively specific for Type 1 DM; they are rare in Type 2 disease,[31] which itself carries a much lower risk of development of DAN.

Clinical and Functional Relationships of Autonomic Nervous Tissue Autoantibodies

There have been several studies attempting to explore the relationship between ANT-autoantibodies and damage to autonomic function. In a series of several similar papers based on cross-sectional observations, Rabinowe and colleagues examined Type 1 DM patients positive and negative for these serological markers for evidence of autonomic dysfunction. The mean postural fall in systolic blood pressure was significantly greater in a group of patients with CF-SG than those without, although none of the antibody-positive patients had orthostatic hypotension according to standard criteria.[29] Only in one patient with orthostatic hypotension were the autoantibodies detected, but in serum samples collected years previously, whereas the sample collected when postural hypotension was recorded was negative. In a study of similar design[30] Type 1 DM patients with CF-VN had lower break index and heart rate variation during deep breathing than those with CF-SG or CF-ADM, thus indicating potential parasympathetic dysfunction associated with CF-VN. A pilot study, involving seven Type 1 DM patients, suggested that CF-ADM and CF-SG are associated with a decreased adrenaline and noradrenaline response to postural change. However, no differences in blood pressure on standing were seen between the antibody-positive and antibody-negative patients.[32]

Subsequently, a comparison was published of ANT autoantibody prevalences in patients carefully selected for fulfilling the diagnosis of DAN on the basis of severe symptoms (diabetic diarrhoea, vomiting due to gastroparesis, bladder paresis, postural hypotension) and functional criteria (deep breathing test, heart rate and blood pressure response on standing, Valsalva manoeuvre) and recruited from a tertiary referral centre, and a group of Type 1 DM patients of comparably long diabetes duration but carefully screened to exclude such complications. Despite small numbers, ANT autoantibodies were exceedingly rare in the uncomplicated group (2/25 patients positive for CF-ADM) but a significant feature of the patients with DAN (33 % positive for at least one autoantibody). Of the three markers, CF-SG was significantly higher in DAN patients when considered alone. Other studies have also indicated a low prevalence of ANT autoantibodies in patients without neuropathy.[27,28]

More recent work also appears to bear out the concept that the association between ANT autoantibodies and autonomic dysfunction is a consistent finding. One of these studies utilized a novel technique to assess sympathetic innervation of the heart in examining two cohorts of patients with newly diagnosed and long-standing Type 1 DM.[27] The patients were assessed for myocardial I^{123}MIBG uptake (metaiodobenzylguanidine, a noradrenalin analogue, the uptake of which is visualized by single-photon emission computed tomography and is indicative of sympathetic innervation), ECG-based cardiac autonomic neuropathy and presence of CF-SG autoantibodies. In the group of long-term diabetic patients the global uptake of the noradrenalin-analogue was negatively correlated with the presence of CF-SG autoantibodies. ECG-based cardiac autonomic neuropathy was present in 22 and absent in 26 patients with long-standing Type 1 DM, of whom 9 (41 %) and 3 (12 %), respectively, were positive for CF-SG autoantibodies, giving a significant correlation between defective cardiac innervation and the presence of cervical ganglia autoantibodies. In the second study of 96 Type 1 DM patients

(41 patients with and 55 without ECG-based cardiac autonomic neuropathy), the frequency of CF-SG autoantibodies was higher in the group with cardiac autonomic neuropathy (34 % vs 18 %), giving borderline significance ($p = 0.06$).[28] At first sight these studies appear at variance with previous work in which ANT autoantibodies appear to be absent in long-standing Type 1 DM patients without cardiac autonomic neuropathy or with formal evidence of autonomic dysfunction but without symptoms.[24] However, it seems likely that the more sophisticated and sensitive techniques used in the more recent studies to investigate cardiac autonomic dysfunction account for this difference in findings. Furthermore, a model may be envisaged in which an active autoimmune process precedes the development of DAN and disappears as autonomic structures are progressively destroyed. These and our own studies favour a stronger disease association for CF-SG and CF-VN autoantibodies than for CF-ADM, which has also been supported by other studies.[33] It should also be noted that Sundkvist and colleagues were unable to find an association between ANT autoantibodies and neuropathic symptoms.[34]

It remains to be established whether the autoantibodies which have been associated with autonomic dysfunction are acting directly or merely reflecting immune-mediated damage to the target tissues. However, Vinik and colleagues have proposed that serum factors themselves may have a detrimental effect on nervous tissues.[35,36] In their study, immunoglobulins from IDDM patients with somatic neuropathy[35] and some with autonomic neuropathy[36] inhibited the growth of a murine neuroblastoma cell line, while sera from patients without this complication did not. Similar studies, using well-characterized autoantibody-positive sera from DAN patients are awaited.

What are the Molecular Targets of Autonomic Nervous Tissue Autoantibodies?

As discussed, all of the ANT autoantibodies described to date have been detected by indirect IFL, which reveals the tissue cells and structures targeted, but gives no information as to the molecular nature of the autoantigens. If the 1970s and 1980s were the years of defining autoantibodies by IFL, the 1990s have seen a number of the molecular targets identified and cloned, most notably in Type 1 DM.[37] Efforts must now be concentrated on defining the molecular targets of anti-ANT autoantibodies, first for the insight it may offer into pathogenesis, and second for the opportunity it affords to utilize modern immunoassays many times more sensitive than IFL to detect the autoantibodies. These advances have been hampered at several levels. The tissues used for the detection of anti-ANT autoantibodies are small structures, and, in the case of the adrenal medulla, have a tendency to autolysis after removal. Acquiring sufficient fresh tissue for detailed study of ganglia and nerves of animal origin is clearly problematic. The autoantibodies themselves are typically of low serum concentration,[31] adding to the problem of defining the antigens. It is probable that the low titre relates to the fact that the target tissues themselves represent a very small proportion of body mass (in general, targets of high titre autoantibodies, such as anti-nuclear autoantibodies in systemic lupus erythematosus and anti-mitochondrial autoantibodies in primary biliary cirrhosis are in great abundance and much easier to define at a molecular level). These restrictions have hampered even the simplest experiments to define the putative molecular weights of the targets, although a recent immunoblotting study along these lines argues for the existence of more than one molecular target of CF-ADM[33] consistent with the definition of different patterns by IFL.[38] In the absence of molecular advances, a degree of guesswork has been employed. For example, on the basis that most endocrine autoantigenic targets are intracellular enzymes, several putative targets have been explored in relation to catecholamine synthesis pathways (e.g. tyrosine hydroxylase, DOPA decarboxylase, dopamine β-hydroxylase, phenylethanolamine N-methyltransferase) and CF-ADM, although without success[33] (Zanone and Peakman, unpublished observations).

Equally, since numerous islet-related autoantigens are shared between the endocrine and nervous systems, such as glutamic acid decarboxylase (GAD), the tyrosine phosphatase like molecule IA-2 and carboxypeptidase H (CPH),[37] they have been considered attractive candidate targets for anti-ANT autoantibodies. However, despite a preliminary early report of autoantibodies to GAD in DAN based on small numbers,[39] several larger studies have now confirmed that there is no relationship between autonomic dysfunction and autoantibodies to GAD.[28,40,41] In the same studies, no relationship between anti-ANT autoantibodies and positivity for either GAD or IA-2 autoantibodies was found, supporting the existence of ANT autoimmunity as an independent phenomenon. As final evidence that GAD is not an ANT autoantigen in DAN, we found no relationship between its subcellular distribution and that of CF-SG and CF-VN autoantibodies, and preincubation of autoimmune sera with brain-derived GAD failed to absorb out ganglion and vagus nerve staining, suggesting in addition that CF-SG and CF-VN may target peripheral nervous system antigens selectively.[42] Using a similar approach for CPH, we found that although it is expressed in the adrenal medulla, specific antiserum for this enzyme has a different cytoplasmic distribution to that of CF-ADM (unpublished observations).

The question remains open, then, as to the molecular nature of the targets of ANT autoantibodies. The fact that acetone fixation fails to abolish staining of the complement-fixing assays used for detection of the ANT antibodies suggests that the autoantigens are protein rather than glycolipid in nature.[42] It has also been demonstrated that the distribution of vagus nerve autoantigens is different from that of myelin components, in keeping with the fact that the vagus nerve has relatively

few myelinated fibres and consistent with an axonal source of antigens indicated by immunofluorescence.[42] A major research goal will be to identify tissues or cell lines which carry the relevant autoantigens but are available more abundantly than ganglia and nerves. Once these are obtained, immunoblotting, immunoprecipitation, and cDNA expression library screening, the mainstays of autoantigen demonstration in recent years, can be fruitfully employed.

Conclusions

The clinical studies argue for a relationship between DAN and autoantibodies to ANT structures, most notably the sympathetic ganglion and vagus nerve. All clinically correlated studies thus far have been cross-sectional and a prospective follow-up study over several years of a large number of case-matched autoantibody-positive and autoantibody-negative patients, recruited soon after DM onset, is required to establish more closely the relationship between autoimmune serological findings and the development of autonomic dysfunction and severe symptomatic DAN, and their power of predicting the complication. Such studies will require enhanced methods of autoantibody detection, preferably using molecularly characterized autoantigens, and it is likely that they will benefit from the type of standardization workshops that have proved so successful for ICA and GAD autoantibodies, with regular exchanges of reference and test sera between laboratories.

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